Brain penetrant small molecule ^{18}F -GnRH receptor (GnRH-R) antagonists: synthesis and preliminary positron emission tomography imaging in rats

Dag E. Olberg ^{a, b}, Nadine Bauer ^c, Kjetil W. Andressen ^{d, e}, Trine Hjørnevik ^f,
Paul Cumming ^{g, h}, Finn O. Levy ^{d, e}, Jo Klaveness ^b, Ira Haraldsen ^g,
and Julie L. Sutcliffe ^{c, i, j, k}.

Keywords: Positron emission tomography, GnRH receptor, Brain imaging
Buchwald-Hartwig, GnRH-R furamide antagonists

^a Norsk medisinsk syklotronsenter AS, Postboks 4950 Nydalen, 0424 Oslo, Norway

^b School of Pharmacy, University of Oslo, P.O.Box 1068 Blindern 0316 Oslo, Norway

^c Department of Biomedical Engineering, University of California Davis, Davis, CA, USA

^d Department of Pharmacology, Faculty of Medicine, University of Oslo and Oslo, University Hospital, Oslo, Norway

^e K.G. Jebsen Cardiac Research Centre and Center for Heart Failure Research, Faculty of Medicine, University of Oslo, Oslo, Norway

^f The Intervention Centre, Oslo University Hospital, PO 4950 Nydalen, 0424 Oslo, Norway

^g Department of Neuropsychiatry and Psychosomatic Medicine, Oslo University Hospital, Oslo, Norway.

^h School of Psychology and Conselling, Queensland University of Technology and QIMR Berghofer, Brisbane, Australia.

ⁱ Division of Hematology/Oncology, Department of Internal Medicine, University of California Davis, Sacramento, California;

^j Center for Molecular and Genomic Imaging, University of California Davis, Davis, California

^k Radiochemistry Research and Training Facility, University of California Davis, Sacramento, California

* Corresponding author:

Dr. Dag E Olberg, Norsk medisinsk syklotronsenter AS, Postboks 4950 Nydalen, 0424 Oslo, Norway and School of Pharmacy, University of Oslo
P.O.Box 1068 Blindern, 0316 Oslo, Norway.

Tel.: [+47] 41517119.

Fax: [+47] 22854402

E-mail address: dag.erlend.olberg@petsenteret.no

Abstract.

Introduction: The gonadotropin releasing hormone receptor (GnRH-R) has a well-described neuroendocrine function in the anterior pituitary. However, little is known about its function in the central nervous system (CNS), where it is most abundantly expressed in hippocampus and amygdala. Since peptide ligands based upon the endogenous decapeptide GnRH do not pass the blood-brain-barrier, we are seeking a high-affinity small molecule GnRH-R ligand suitable for brain imaging by positron emission tomography. We have previously reported the radiosynthesis and *in vitro* evaluation of two novel [^{18}F]fluorinated GnRH-R ligands belonging to the furamide class of antagonists, with molecular weight less than 500 Da. We now extend this work using palladium coupling for the synthesis of four novel radioligands, with putatively reduced polar surface area and hydrophilicity relative to the two previously described compounds, and report the uptake of these ^{18}F -labelled compounds in brain of living rats.

Methods: We synthesized reference standards of the small molecule GnRH-R antagonists as well as mesylate precursors for ^{18}F -labelling. The antagonists were tested for binding affinity for both human and rat GnRH-R. Serum and blood stability *in vitro* and *in vivo* was studied. Biodistribution and PET imaging studies were performed in male rats in order to assess brain penetration *in vivo*.

Results: A palladium coupling methodology served for the synthesis of four novel fluorinated furamide GnRH receptor antagonists with reduced heteroatomic count. Radioligand binding assay assays *in vitro* revealed subnanomolar affinity of the new fluorinated compounds for both human and rat GnRH-R. The ^{18}F -GnRH antagonists were synthesized from the corresponding mesylate precursors in 5-15 % overall radiochemical yield. The radiolabeled compounds demonstrated good *in vivo* stability. PET imaging with the ^{18}F -radiotracers in naive rats showed good permeability into brain and rapid washout, but absence of discernible specific binding *in vivo*.

Conclusions:

The novel small molecule ^{18}F -fluorinated GnRH-R antagonist compounds show high receptor affinity *in vitro*, and may prove useful for quantitative autoradiographic studies *in vitro*. The compounds were permeable to the blood brain barrier, but nonetheless failed to reveal significant specific binding in brain of living rats. Nonetheless, our approach

may serve as a foundation for designing PET ligands suitable to image the GnRH-R distribution in brain.

Abbreviations: Positron emission tomography (PET) G-protein coupled receptor (GPCR), gonadotropin releasing hormone receptor (GnRH-R)

1. Introduction

The 60 kDa gonadotropin release hormone receptor (GnRH-R) is a seven transmembrane G-protein-coupled receptor (GPCR), but lacks the usual intracellular C-terminal that mediates agonist-induced internalization of rhodopsin-like receptors [1]. The receptor is highly conserved, with more than 80% overall identity in mammals [2], and serves a pivotal function in the hypothalamic-pituitary-gonadal (HPG) reproductive endocrine axis. The endogenous ligand GnRH, formerly known as luteinizing hormone releasing hormone (LHRH), is a linear decapeptide with amino acid sequence pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂. GnRH is produced in specific neurons of the anterior hypothalamus, and regulates the release of gonadotropin from cells in the anterior pituitary bearing a high density of GnRH-R; while the natural peptide GnRH peptide is degraded very rapidly *in vivo*, peptidase-resistant derivatives with antagonist or agonist properties are widely used in medicine to treat precocious puberty and other endocrine disorders through a net inhibition of the HPG axis [3]. However, GnRH-Rs are also present in a variety of non-reproductive tissues, including heart, liver, kidney, skin and skeletal muscle, and likewise in the central nervous system; autoradiographic studies *in vitro* indicate moderately high expression of GnRH-R in the hippocampal formation ($B_{\max}$ 11.6 fmol/mg protein in the CA3 region), and lesser amounts in the amygdala and medial hypothalamus [4, 5]. Nothing is known about the function of these receptors in the central nervous system (CNS), but they seem poised to mediate cognitive and affective aspects of reproductive cycle [6]; their association with the hippocampus presents the GnRH-R as a potential therapeutic target for treatment of Alzheimer's dementia.

Non-peptide orally-active GnRH-R antagonists have recently been developed in order to avoid the side-effects associated with peptide agonist derivatives, which require subcutaneous administration [7]. Since the GnRH peptides (MW $\approx$ 1200) have very limited penetration of the blood-brain barrier (BBB), we anticipated that small molecular weight GnRH-R ligands might serve as tracers to visualize intracerebral GnRH-Rs *in vivo* by positron emission tomography (PET) [8]. We have recently reported on the synthesis of **IP1** and **IP2**, two GnRH-R antagonists based on the furamide lead structure, which are amenable for labeling with fluorine-18 for use in PET investigations (Fig. 1)

[9]. However, these PET ligands, despite their low molecular weight (≈ 500 Da), seemingly displayed little or no brain uptake in living rats. In an extension of that work, and in order to improve further the GnRH-R affinity, we now report the synthesis of four highly potent furamide GnRH-R antagonists, also suitable for labeling with fluorine-18. With an aim to optimize brain penetration, we incorporated new motifs intended to reduce topological polar surface area (tPSA) and increase lipophilicity compared to our previously reported compounds of this class.

Fig. 1.

In particular, we sought to reduce the tPSA of our previous compounds by replacing the ether-link bridging the indane and furan motifs with a methylene group, and by replacing the heteroatomic pyrimidine system with a benzene ring to improve brain penetration, while retaining high GnRH-R affinity. The linker was conserved in new analogs, as it was found to be important for high GnRH-R affinity, and also serves as a convenient introduction point for [^{18}F]fluoride from the methanesulfonic ester precursor [9]. Furthermore, we also replaced the indane system of **IP1** and **IP2** with a tetraline system, affording a more straightforward synthetic route, while unlikely to reduce binding affinity of the resultant GnRH-R antagonists [10]. To explore effects of linker length, we tested compounds with an additional carbon in form of an aminopropyl linker. *In silico* calculations of tPSA and log P of these putatively new compounds, although imperfect predictors of BBB permeability, indicated significantly reduced tPSA and increased lipophilicity, with negligible increase of the molecular weight with respect to the previously reported **IP1** and **IP2** (Table 1).

Table 1.

2. Material and methods

General Information: Solvents and chemicals were purchased from Aldrich (Milwaukee, WI), Fisher Scientific or VWR unless stated otherwise. Sep-Pak SPE cartridges were obtained from Waters (Milford, USA) and ^{18}F Trap & Release Columns were purchased from ORTG, Inc. (Oakdale, USA). RP-HPLC was performed using Beckman-Colter (Brea, USA) chromatography systems equipped with Jupiter Proteo C-12 columns (250 x

4.6 mm, 4 mm, Phenomenex, Torrance, USA) and single wavelength or diode array UV detectors (set to 254 nm) connected in series to a Bioscan Flow Count photo multiplier tube (PMT) (Bioscan, Washington DC, USA). Mass spectrometry analysis was performed using a Thermo Electron LTQ-Orbitrap Hybrid MS spectrometer. NMR spectra were recorded using a Bruker Avance 500 or 600 spectrometer. [^{18}F]Fluoride was produced from the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction on [^{18}O]H₂O purchased from Marshall Isotopes Ltd. (Tel Aviv, Israel) using a CTI RDS 111 negative ion cyclotron (Knoxville, USA). Total radioactivity was measured with a Capintec dose calibrator.

HPLC methods: Reversed-phase HPLC was used to purify and analyze the products; solvent A: 0.05% TFA in water (v/v); solvent B: acetonitrile. The UV detector (UV, 254 nm) and radioactivity detector (PMT) connected in series accounts for the slight difference in retention times for corresponding ^{18}F - and ^{19}F -compounds. Analytical HPLC system A: Phenomenex Jupiter 4 μ Proteo 90 Å column (250 $\times$ 4.6 mm, 4 μm), solvent B isocratic 50% for 2 min, then linear gradient to 90% over 20 min, flow rate 1.5 mL min⁻¹; Semi-preparative HPLC system B: Phenomenex Jupiter 10 μ Proteo 90 Å (250 $\times$ 10 mm, 10 μm), solvent B isocratic 50% for 2 min, then linear gradient to 90% over 30 min, flow rate 3 mL min⁻¹. Sep-Pak SPE cartridges were precondition according to the manufacturers recommendations. Correct identity of the ^{18}F -radiolabeled products were characterized by co-injection with the authentic reference ^{19}F -compounds.

Binding studies: Radioligand binding studies *in vitro* were performed with 0.18-0.32 nM [^{125}I]LHRH-[D-Trp⁶] ([^{125}I]Triptorelin) (PerkinElmer, Boston, USA) and a range of concentrations of the four test compounds in a membrane preparation from cells expressing the human type I GnRH-R or rat GnRH-R, as previously described [9].

Partition coefficients: To measure the octanol/water partition coefficients of the four ^{18}F -labelled compounds, approximately 10 kBq of each tracer in 50 μL PBS was diluted in 450 μL of PBS pH 7.4, which was mixed with 500 μL of *n*-octanol by vortexing in an Eppendorf tube for three minutes, in triplicate for each compound. The tubes were centrifuged (10,300 rpm;) for six minutes, and 100 μL portions of the PBS and *n*-octanol

phases were carefully transferred to separate tubes for measurement of their radioactivity content in a γ -counter.

Serum stability analysis. Approximately 5 MBq of each ^{18}F -compound in saline (50 μL) was added to freshly prepared rat serum (400 μL) in Eppendorf tubes, which were incubated at 37 °C. After 1 and 2 h, ice-cold ethanol (400 μL) was added, and the mixtures were centrifuged at 13,400 rpm for 10 min. The supernatant was diluted with Solvent A (1:1) and analyzed by radio-HPLC.

Ex vivo blood and urine analysis: Following intravenous tracer administration, rats were euthanized, for collection of urine samples (1 and 2 h) and blood (1 h). Whole blood was centrifuged to separate the serum. Ice-cold ethanol (1 mL) was added to aliquots of either urine or serum and centrifuged at 13,400 rpm for 10 min. The supernatant was diluted with Solvent A and analyzed by radio-HPLC.

In vivo experimental methods (biodistribution): Male Sprague Dawley rats (13 weeks old) were purchased from Charles River Laboratories (Wilmington, USA) and handled in accordance with a protocol approved by the University of California, Davis, Animal Use and Care Committee. Food and water were available ad libitum.

For biodistribution studies, rats ($n=3/\text{compound}$) were anesthetized with 2% isoflurane in oxygen (0.8 L/min), and injected intravenously (i.v.) with 9 to 15 MBq of [^{18}F]SB1, 2, 3, or 4 via a catheter placed in a tail vein. After a one-hour conscious uptake period rats were re-anaesthetized and killed by decapitation. Organ samples were resected, rinsed with PBS, weighed, and their radioactivity content measured in a Wizard 1470 gamma counter (Perkin-Elmer, Waltham, USA). Calibrated radioactivity concentrations are expressed as percent of injected dose per gram of sample (% ID g^{-1}), decay-corrected to the time of tracer injection.

Small animal PET imaging: Rats were anesthetized with 2-3% isoflurane in oxygen (0.8 L/min), and placed in a head-first prone position with head forward in the aperture of an Inveon small animal PET scanner (Siemens Medical Solutions USA, Knoxville, TN). After placement of a tail vein catheter, each formulated radiotracer [^{18}F]SB1, 2, 3, and 4 (23-30 MBq) was injected i.v. in single rats, at which time a dynamic emission sequence was initiated, consisting of 29 frames (6 $\times$ 30 s, 6 $\times$ 60 s, 7 $\times$ 180 s, 6 $\times$ 300 s, 4 $\times$ 900 s), to a

total of 120 min. Anesthesia was maintained at 1.5 to 2.0% isoflurane in oxygen (0.8 L/min) for the duration of the scan. Prior to each emission recording, a cobalt-57 transmission scan (900 s) had been acquired for attenuation correction. CT scans (effective pixel size: 99.48 μm) for anatomical reference were acquired on the Inveon SPECT/CT system (Siemens Medical Solutions USA, Knoxville, TN). Fiducial markers attached to the animal bed were used for subsequent alignment of the PET and CT scans. PET list mode data were reconstructed with an OSEM3D fastMAP algorithm into a 256×256 matrix (Pixel size: 0.39 mm) and attenuation corrected using the Inveon Acquisition Workplace software package (Siemens Medical Solutions USA, Knoxville, TN). The dynamic emission images were registered to an MR rat brain template using PMOD analysis software (PMOD Technologies Ltd, Switzerland). The mean %ID g^{-1} during the initial three minutes uptake phase and in the late interval of 45-120 minutes were calculated. We determined an image derived arterial input function (IDIF) based on methods reported previously [11]. To this end, we manually placed a spherical VOI of 1 mm diameter on the left ventricle of the heart, identified from the blood-pool image in the first four dynamic image frames. The entire IDIF was extracted from the 120 min dynamic sequence. Using this IDIF, we then analyzed the cerebral uptake during the initial two min by linear graphic analysis, plotting the distribution volume in measured in a VOI for entire forebrain as a linear function of the normalized input (i.e. Gjedde Patlak analysis), as described previously [11]. Here the ordinate intercept corresponds to the apparent plasma volume (V_p ; ml g^{-1}) and the initial slope during the first two minutes is the unidirectional blood-brain clearance (K_I ; $\text{ml g}^{-1} \text{min}^{-1}$). Additional voxelwise analysis by a 1-tissue compartment model (1TCM) was undertaken in PMOD to depict the equilibrium distribution volume (V_T ; ml g^{-1}) for each tracer, also using the IDIF. Furthermore, we applied the 1TCM model to dynamic time-activity curves from a VOI placed on the bilateral hippocampus, in order to estimate the magnitudes of K_I ; k_2 (min^{-1} ; the brain-blood clearance rate constant) and V_T in that structure, where GnRH-R is most abundant [12].

Chemistry.

5-bromo-1,3-dimethoxy-2-nitrobenzene (5). **5** was prepared according to J-M Vernier *et al.* [13]. ¹H NMR (600 MHz, DMSO): δ 7.17 (s, 2H), δ 3.89 (s, 6H); ¹³C NMR (151 MHz, DMSO) δ 151.51, 130.33, 124.87, 109.22, 108.88, 57.32.

1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene (1). Tetralin **1** was prepared according to Parlow JJ [14].

¹H NMR (600 MHz, CDCl₃): δ 7.25 (d, 1H, *J* = 8 Hz), δ 6.98 (d, 1H, *J* = 8 Hz), δ 6.90 (bs, 1H), δ 2.76 (t, 2H), δ 2.31 (t, 3H), δ 1.83 (m, 2H), δ 1.69 (m, 2H), δ 1.31 (s, 6H); ¹³C NMR (151 MHz, CDCl₃): δ 143.02, δ 136.13, δ 134.71, δ 129.74, δ 126.86, δ 126.69, δ 39.65, δ 33.68, δ 32.05, δ 32.05, δ 30.87, δ 20.96, δ 19.98..

5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxylic acid (3). To a solution containing **1** (0.5 g, 2.87 mmol) and methyl 5-(chloromethyl)-2-furoate (0.42 g, 2.39 mmol) in nitro methane (10 ml) was added aluminum trichloride (0.383 g, 2.87 mmol) slowly under N₂-gas. The solution was stirred at room temperature for 48 h. The reaction was quenched with ice-cold water and crude product extracted with ethyl acetate (2 ×100 ml). The combined organic phases were washed with brine and dried over MgSO₄ and concentrated under vacuum. The crude product was purified by silica gel chromatography using hexanes/ethyl acetate (19/1 v/v) to afford 0.302 g (40%) of a mixture of **2a** and **2b** (7- and 5-regioisomers) as viscous oil. **2a** and **2b** were not isolated as a pure compound and fully characterized but isolated after alkaline hydrolysis of the methyl ester.

The mixture of **2a** and **2b** was dissolved in THF (10 mL) and 4 M NaOH (10 mL) was added. The reaction mixture was stirred overnight and quenched with 1M HCl until an acidic pH. The reaction was extracted with dichloromethane and concentrated *in vacuo*. The mixture of isomers was dissolved in 1:1 mixture of mobile phase A and B and subjected to reversed phased preparative HPLC using an isocratic method (61 % solvent B in A at a flow rate of 7 ml min⁻¹ using a preparative reversed phased C18 column (Phenomenex Jupiter C18 10 μm, 250 × 21.2 mm) to afford 100 mg of isomerically pure **3**. ¹H NMR (600 MHz, CDCl₃): δ 7.22 (d, *J* = 3.5, 1H), δ 7.13 (s, 1H), δ 6.87 (s, 1H), δ 5.97 (d, *J* = 3.5, 1H), δ 4.00 (s, 2H), δ 2.27 (t, 2H), δ 2.20 (s, 3H), δ 1.78 (m, 2H), δ 1.64 (m, 2H) δ 1.25 (s, 6H); ¹³C NMR (151 MHz, CDCl₃): δ 163.35, δ 161.52, δ 143.97, δ 142.37, δ 135.28, δ 133.41, δ 132.10, δ 131.15, δ 128.27, δ 121.70, δ 109.28, δ 39.42, δ

33.65, δ 32.75, δ 32.00 (s), δ 32.00 (s) δ 30.40, δ 19.86, δ 18.98; HMBC and HSQC confirm the correct regioisomer; HRESIMS $[M + H]^+$ m/z 299.1645 (calcd for $C_{19}H_{22}O_3$, 299.1642).

3-((3,5-dimethoxy-4-nitrophenyl)amino)propan-1-ol (6e). CS_2CO_3 (684 mg, 2.1 mmol), ($\pm$ BINAP) 2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene (56 mg, 0.1 mmol) and palladium(II) acetate (6.7 mg, 0.03 mmol) in 1 mL 1,4-dioxane was stirred under argon in a 25 mL Schlenk tube at 70 °C for 10 min acquiring a deep red color. The bromide, **5**, (300 mg, 1.2 mmol) and 3-amino-1-propanol (135 mg, 1.8 mmol) in 3 mL 1,4-dioxane were added to the catalytic complex under a counter stream of argon. The sealed reaction mixture was stirred for 16 h at 110 °C, cooled and diluted with 10 mL ethyl acetate. After filtration through celite and concentration, the residue was purified by flash chromatography (ethyl acetate) affording **6e** as a yellow oil (180 mg, 58%). 1H NMR (600 MHz, $CDCl_3$): δ 5.80 (s, 2H), δ 3.82 (t, $J = 5.7$, 2H), δ 3.80 (s, 6H), δ 3.30 (t, $J = 6.5$, 2H), δ 1.89 (p, $J = 6.3$, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 154.40, δ 150.98, δ 123.73, δ 88.74, δ 61.20, δ 56.37, δ 42.00, δ 31.30; HRESIMS $[M + H]^+$ m/z 257.1128 (calcd for $C_{11}H_{16}N_2O_5$, 257.1132).

3-((4-amino-3,5-dimethoxyphenyl)amino)propan-1-ol (7c). **6e** (100 mg, 0.4 mmol), $Fe_{(s)}$ (125 mg), NH_4Cl (125 mg) in a mixture of $H_2O/MeOH$ (12 mL, 2:10) was stirred vigorously at room temperature for 2 h. Solids were filtered off and the filtrate concentrated. The residue was dissolved in dichloromethane and the ammonium chloride was filtered off. After concentration the residue was purified by flash chromatography (ethyl acetate, neat) affording **7c** as black viscous oil (25 mg, 28%). The compound was highly unstable and was used immediately in the following reaction. NMR was not feasible.

HRESIMS $[M + H]^+$ m/z 227.1389 (calcd for $C_{11}H_{18}N_2O_3$, 227.1390).

N-(3-fluoropropyl)-3,5-dimethoxy-4-nitroaniline (6f). CS_2CO_3 (500 mg, 1.5 mmol), ($\pm$ BINAP) 2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene (38 mg, 0.06 mmol) and palladium(II) acetate (5 mg, 0.02 mmol) in 1 mL 1,4-dioxane was stirred in a 25 mL Schlenk tube at 70 °C for 10 min under an argon atmosphere, during which the solution acquired a deep red color. Aryl bromide, **5**, (200 mg, 0.8 mmol) and 3-fluoropropylamine hydrochloride (132 mg, 1.2 mmol) in 2 mL 1,4-dioxane was added to the

catalytic complex under a counter stream of argon. The sealed reaction mixture was stirred for 5 h at 80 °C, cooled and diluted with 10 ml ethyl acetate. After filtration through celite and concentration, the residue was purified by flash chromatography (40 % ethyl acetate in hexanes) affording **6f** as an oil (180 mg, 44 %). ¹H NMR (600 MHz, CDCl₃): δ 5.76 (s, 2H), δ 4.62 (t, *J* = 5.5, 1H), δ 4.55 (t, *J* = 5.5, 1H), δ 3.80 (s, 6H), δ 3.33 (t, *J* = 6.7, 2H), δ 2.03 (m, 1H), δ 1.99 (m, 1H); ¹³C NMR (151 MHz, CDCl₃): δ 154.4, 151.3, 88.3, , 82.8, 81.74, 56.4, 40.4, 30.3; HRESIMS [M + H]⁺ *m/z* 259.1087 (calcd for C₁₁H₁₅FN₂O₄, 259.1089).

N¹-(3-fluoropropyl)-3,5-dimethoxybenzene-1,4-diamine (7d). **6f** (92 mg, 0.36 mmol), Fe_(s) (200 mg), EtOH (3 mL) and saturated NH₄Cl (3 mL) was stirred vigorously at RT for 3 h. The spent iron was filtered off and the filtrate concentrated neutralized a saturated sodium bicarbonate solution and extracted with dichloromethane. After concentration the residue was purified by flash chromatography (ethyl acetate) affording **7d** as an oil (64 mg, 78%). ¹H NMR (600 MHz, CDCl₃): δ 5.87 (s, 2H), δ 4.63 (t, *J* = 5.5, 1H), δ 4.55 (t, *J* = 5.5, 1H), δ 3.78 (s, 6H), δ 3.75 (t, 2H), δ 1.99 (m, 2H); ¹³C NMR (151 MHz, CDCl₃): δ 148.14, 140.28, 119.73, 98.47, 83.12, 82.03, 55.95, 55.84; HRESIMS [M + H]⁺ *m/z* 229.1350 (calcd for C₁₁H₁₇FN₂O₂, 229.1347).

5-(2-(tert-butoxy)ethoxy)-1,3-dimethoxy-2-nitrobenzene (6b). To a stirred solution of Cs₂CO₃ (391 mg, 1.2 mmol), 5-di(1-adamantylphosphino)-1-(1,3,5-triphenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazole (11 mg, 0.017 mmol) and palladium(II) acetate (1.8 mg, 1 mol %) in 2 mL dry toluene in a 25 mL Schlenk tube at 80 °C was added aryl bromide **5**, (200 mg, 0.8 mmol) and 2-tert-butoxyethanol (284 mg, 2.4 mmol) under an argon atmosphere. The sealed reaction mixture was stirred at 80 °C overnight. After cooling, the reaction mixture was diluted with 10 ml ethyl acetate and filtered through celite and concentrated. The residue was purified by flash chromatography (20 % ethyl acetate in hexanes) affording **6b** as an oil (156 mg, 68 %). ¹H NMR (600 MHz, CDCl₃): δ 6.17 (s, 2H), δ 4.11 (t, *J* = 5.3, 2H), δ 3.84 (s, 6H), δ 3.72 (t, *J* = 5.3, 2H), δ 1.24 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 161.78, 153.39, 91.68, 73.71, 70.06, 68.80, 60.43, 56.54, 27.63; HRESIMS [M + H]⁺ *m/z* 300.1442 (calcd for C₁₄H₂₁NO₆, 300.1442)

2-(3,5-dimethoxy-4-nitrophenoxy)ethanol (6c). The aryl ether **6b** (60 mg, 0.2 mmol) was stirred for 48 h in a solution of 85 % phosphoric acid (3 mL) and toluene (1 mL).

The reaction mixture was neutralized and extracted with dichloromethane (3×10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to afford **6c** as an oil (39 mg, 80%). ^1H NMR (600 MHz, CDCl_3) δ 6.15 (s, 2H), 4.11 (t, $J = 4.5$, 2H), 3.99 (t, $J = 4.5$, 2H), 3.85 (s, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 161.33, 153.43, 106.13, 91.44, 69.86, 61.31, 56.56; HRESIMS $[\text{M} + \text{H}]^+$ m/z 244.0812 (calcd for $\text{C}_{10}\text{H}_{13}\text{NO}_6$, 244.0816).

4-(2-(tert-butoxy)ethoxy)-2,6-dimethoxyaniline (7a). **6b** (156 mg, 0.52 mmol), $\text{Fe}_{(\text{s})}$ (200 mg) in EtOH (4 mL) and saturated NH_4Cl (3 mL) was stirred vigorously at room temperature for 3 h. The spent iron was filtered off, and the filtrate was neutralized with 1 M NaOH and then diluted with 30 mL H_2O . The aqueous phase was extracted with dichloromethane (3×10 mL) and ethyl acetate (3×10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated affording **7a** as an oil (139 mg, 99 %). ^1H NMR (600 MHz, CDCl_3): δ 6.18 (s, 2H), 4.06 (t, $J = 5.4$, 2H), 3.86 (s, 6H), 3.71 (t, $J = 5.4$, 2H), 1.24 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 157.64, 154.29, 105.34, 91.98, 73.64, 68.62, 60.53, 56.34, 27.63; HRESIMS $[\text{M} + \text{H}]^+$ m/z 270.1702 (calcd for $\text{C}_{14}\text{H}_{23}\text{NO}_4$, 270.1700).

5-(2-fluoroethoxy)-1,3-dimethoxy-2-nitrobenzene (6d). Alcohol, **6c**, (44.5 mg, 0.18 mmol) in dry dichloromethane (4 mL) was cooled to 0 °C. Diethylaminosulfur trifluoride (DAST) (30 mg, 23 mmol) was added dropwise over 1 min. The reaction mixture was first stirred 1 h at 0 °C then allowed to reach room temperature before quenched with saturated sodium bicarbonate solution. The reaction mixture was extracted with dichloromethane (3×10 mL), the combined organic phases were washed with water and dried over MgSO_4 . Following concentration the residue was purified by flash chromatography (60% ethyl acetate in hexanes) affording **6d** as an oil (30 mg, 65 %). ^1H NMR (600 MHz, CDCl_3): δ 6.16 (s, 2H), 4.81 (m, 1H), 4.73 (m, 1H), 4.27 (m, 1H), 4.22 (m, 1H), 3.86 (s, 6H); ^{13}C NMR (151 MHz, CDCl_3): δ 161.04, 153.47, 91.62, 82.22, 81.08, 67.85, 67.71, 56.61; HRESIMS $[\text{M} + \text{H}]^+$ m/z 246.0769 (calcd for $\text{C}_{10}\text{H}_{12}\text{FNO}_5$, 246.0773).

4-(2-fluoroethoxy)-2,6-dimethoxyaniline (7b). The nitrophenyl derivative, **6d**, (30 mg, 0.12 mmol), $\text{Fe}_{(\text{s})}$ (120 mg) in EtOH (3 mL) and saturated NH_4Cl (3 mL) was stirred

vigorously at room temperature for 3 h. Solids were filtered off and the filtrate was neutralized with sodium bicarbonate and diluted with 30 mL H₂O. The aqueous phase was extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried over Na₂SO₄ and concentrated, affording **7b** as an oil (18 mg, 68 %). ¹H NMR (600 MHz, CDCl₃): δ 6.16 (s, 2H), 4.80 (m, 1H), 4.72 (m, 1H), 4.26 (m, 1H), 4.21 (m, 1H), 3.85 (s, 6H); ¹³C NMR (151 MHz, CDCl₃): δ 151.36, 148.09, 119.65, 92.73, 82.30 (d, ¹J_{CF} = 171 Hz), 68.45 (d, ²J_{CF} = 20.3 Hz), 56.10; HRESIMS [M + H]⁺ m/z 216.1031 (calcd for C₁₀H₁₄FNO₃, 216.1031).

N-(4-((3-hydroxypropyl)amino)-2,6-dimethoxyphenyl)-5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamide (8a). To a stirred vial charged with acid **3** (26 mg, 0.1 mmol, 1 eq.) 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (34 mg, 1 eq), *N,N*-diisopropylethylamine (DIPEA) (37 μL, 3 eq.) in DMF (1 mL) was allowed to stand for 5 min before amine **7c** was added (24 mg, 0.09 mmol). After 1 h reaction time HPLC indicated a complete reaction (full consumption of acid). The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 5 mL). The combined organic phases were washed with water and dried over Na₂SO₄. Following removal of organic solvent *in vacuo* the residue was purified by flash chromatography (ethyl acetate, neat), affording the alcohol **8a** as a yellowish solid (25 mg, 54 %). ¹H NMR (600 MHz, CDCl₃): δ 8.02 (s, 1H), 7.10 (s, 2H), 7.09 (d, *J* = 3.5, 1H), 6.88 (s, 1H), 6.55 (s, 1H), 6.01 (d, *J* = 3.5, 1H), 3.84 (t, 2H), 3.80 (s, 6H), 3.48 (s, 2H), 3.41 (t, 2H), 2.71 (t, 2H), 2.08 (s, 3H), 2.01 (m, 2H), 1.79 (m, 2H), 1.64 (m, 2H), 1.24 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 175.33, 162.84, 157.76, 156.41, 146.55, 143.93, 135.18, 133.31, 132.54, 131.11, 128.00, 116.55, 109.28, 96.11, 60.30, 56.41, 39.43, 36.70, 33.64, 32.51, 31.99, 31.64, 30.38, 20.75, 19.87, 19.00; HRESIMS [M + H]⁺ m/z 507.2854 (calcd for C₃₀H₃₈N₂O₅, 507.2854).

N-(4-((3-hydroxypropyl)amino)-2,6-dimethoxyphenyl)-5-((3,3,6-trimethyl-2,3-dihydro-1H-inden-5-yl)oxy)furan-2-carboxamide (8b). To a stirred vial charged with acid **9** (12 mg, 0.04 mmol, 1 eq.) HATU (15.9 mg, 1 eq), DIPEA (*N,N*-diisopropylethylamine) (22 μL, 3 eq.) in DMF (1 mL) was allowed to stand for 5 min

before amine **7c** was added (11 mg, 0.05). HPLC indicated a complete reaction (full consumption of acid) after 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 5 mL). The combined organic phases were washed with water and dried over Na₂SO₄. Following removal of organic solvent *in vacuo* the residue was purified by flash chromatography (ethyl acetate, neat), affording **8b** as a yellowish solid (16 mg, 77 %). ¹H NMR (600 MHz, CDCl₃): δ 7.10 (s, 2H), 7.09 (d, 1H), 6.88 (s, 1H), 6.55 (s, 1H), 5.29 (d, 1H), 3.89 (t, 2H), 3.80 (s, 6H), 3.41 (t, 2H), 2.71 (t, 2H), 2.84 (t, 2H), 2.22 (s, 3H), 1.94 (t, 2H), 1.76 (m, 2H), 1.23 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 162.56, 159.94, 156.18, 152.54, 147.80, 140.68, 134.98, 127.52, 122.67, 115.85, 113.44, 108.91, 104.93, 87.46, 58.54, 56.57, 44.33, 41.91, 29.79, 28.74, 16.43;

HRESIMS [M + H]⁺ m/z 495.2479 (calcd for C₂₈H₃₄N₂O₆, 495.2490).

N-(4-(2-(tert-butoxy)ethoxy)-2,6-dimethoxyphenyl)-5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamide (8c). To a stirred vial charged with acid **3** (24 mg, 0.08 mmol, 1 eq.) HATU (32 mg, 1 eq), DIPEA (*N,N*-diisopropylethylamine (44 μL, 3 eq.) in DMF (1 mL) was allowed to stand for 5 min before amine **7a** (23 mg, 0.09 mmol) was added. HPLC indicated a complete reaction (full consumption of acid) after 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate (3 × 5 mL). The combined organic phases were washed with water and dried over Na₂SO₄. Following removal of organic solvent *in vacuo*, the residue was purified by flash chromatography (1:1 Ethyl acetate/hexanes), affording **8c** as a glassy solid (22 mg, 48 %). ¹H NMR (600 MHz, CDCl₃) δ 7.26 (d, 1H), 7.10 (s, 1H), 6.87 (s, 1H), 6.22 (s, 2H), 5.99 (d, 1H), 4.09 (t, 2H), 3.96 (s, 2H), 3.79 (s, 6H), 3.72 (t, 2H), 2.71 (t, 2H), 2.24 (s, 3H), 1.79 (m, 2H), 1.64 (m, 2H), 1.24 (s, 6H), 1.24 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 159.53, 156.46, 143.87, 135.08, 133.35, 132.72, 131.07, 128.01, 116.11, 109.19, 106.57, 92.00, 73.58, 68.51, 60.56, 56.19, 39.45, 33.64, 32.52, 32.00, 30.38, 27.66, 19.88, 19.04; HRESIMS [M + H]⁺ m/z 550.3145 (calculated for C₃₃H₄₃NO₆, 550.3163).

N-(4-(2-(tert-butoxy)ethoxy)-2,6-dimethoxyphenyl)-5-((3,3,6-trimethyl-2,3-dihydro-1H-inden-5-yl)oxy)furan-2-carboxamide (8d). A stirred vial charged with the acid chloride of **9** (34 mg, 0.11 mmol, 1.2 eq.) and DIPEA (*N,N*-diisopropylethylamine (50 μL, 3 eq.) in dry dichloromethane (1 mL) was cooled to 0 °C; to this the amine **7a** (25

mg, 0.09 mmol) in dichloromethane (0.5 mL) was added dropwise. HPLC indicated a complete reaction (full consumption of amine) after 5 h reaction time. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with 0.1 M HCl, brine and water. The organic phase was dried over Na₂SO₄. Following removal of organic solvent *in vacuo*, the residue was purified by flash chromatography (7:3 ethyl acetate/hexanes), affording **8d** as a glassy solid (30 mg, 62 %). ¹H NMR (600 MHz, CDCl₃) δ 7.20 (bs, 1H), 7.12 (d, *J* = 3.5, 1H), 7.06 (s, 1H), 6.85 (s, 1H), 6.22 (s, 2H), 5.29 (d, *J* = 3.5, 1H), 4.09 (t, *J* = 5.6, 2H), 3.80 (s, 6H), 3.72 (t, *J* = 5.6, 2H), 2.85 (t, *J* = 7.2, 2H), 2.25 (s, 3H), 1.94 (t, *J* = 7.2, 2H), 1.25 (s, 9H), 1.22 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 159.52, 156.47, 152.38, 152.10, 140.01, 127.37, 126.82, 112.70, 106.64, 92.04, 87.94, 73.56, 68.49, 60.55, 56.14, 44.25, 41.88, 29.69, 28.67, 27.65, 16.01; HRESIMS [M + H]⁺ *m/z* 538.2803 (calcd for C₃₁H₃₉NO₇, 538.2800).

***N*-(4-(2-hydroxyethoxy)-2,6-dimethoxyphenyl)-5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamide (10a).** **8c** (22 mg, 0.04 mmol) was stirred vigorously at 50 °C for 24 h in a solution of 85% phosphoric acid (2 mL) and toluene (1 mL). The reaction mixture was quenched with 0.1 M NaOH, diluted with (10 mL) water and extracted with dichloromethane (3 × 5 mL). The organic phases were combined, washed with water (5 mL) and dried over Na₂SO₄. After removal of organic solvent *in vacuo*, **10a** was used further without purification. ¹H NMR (600 MHz, CDCl₃) δ 7.47 (bs, 1H), 7.11 (d, 1H), 6.89 (s, 1H), 6.21 (s, 1H), 6.05 (d, 1H), 5.30 (s, 2H), 4.08 (t, 2H), 4.00 (t, 2H), 3.99 (s, 2H), 3.83 (s, 6H), 2.72 (t, 2H), 2.25 (s, 3H), 1.79 (m, 2H), 1.65 (m, 2H), 1.26 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 158.31, 143.94, 135.22, 133.28, 131.12, 127.99, 91.90, 61.85, 39.42, 33.62, 32.00, 30.35, 19.84, 19.05; HRESIMS [M + H]⁺ *m/z* 494.2512 (calcd for C₂₉H₃₅NO₆, 494.2537).

***N*-(4-(2-hydroxyethoxy)-2,6-dimethoxyphenyl)-5-((3,3,6-trimethyl-2,3-dihydro-1H-inden-5-yl)oxy)furan-2-carboxamide (10b).** **8d** (30 mg, 0.06 mmol) was stirred vigorously at 50 °C for 24 h in a solution of 85% phosphoric acid (2 mL) and toluene (1 mL). The reaction mixture was quenched with 0.1 M NaOH, diluted with (10 mL) water and extracted with dichloromethane (3 × 5 mL). The organic phases were combined and washed with water (5 mL) and dried over Na₂SO₄. After removal of organic solvent *in vacuo*, the **10b** was used further without purification. ¹H NMR (600 MHz, CDCl₃) δ 7.17

(d, 1H), 7.01 (s, 1H), 6.95 (s, 1H), 6.17 (s, 2H), 5.30 (d, 1H), 4.08 (t, 2H), 3.98 (t, 2H), 3.79 (s, 6H), 3.72 (t, 2H), 2.20 (s, 3H), 1.88 (t, 2H), 1.21 (s, 6H); ^{13}C NMR (151 MHz, CDCl_3) δ 174.19, 172.32, 168.58, 167.72, 152.78, 152.06, 134.78, 126.75, 121.58, 108.89, 103.13, 102.01, 91.65, 63.89, 61.56, 57.22, 56.68, 56.11, 44.11, 42.27, 42.03, 31.37, 29.48, 28.73, 16.02; HRESIMS $[\text{M} + \text{H}]^+$ m/z 482.2166 (calcd for $\text{C}_{27}\text{H}_{31}\text{NO}_7$, 482.2174).

3-((3,5-dimethoxy-4-(5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamido)phenyl)amino)propyl methanesulfonate (SBP-1). To a stirred vial under an argon atmosphere cooled to 0 °C containing **8a** (25 mg, 0.05 mmol), triethylamine (21 μL , 0.15 mmol) in dichloromethane (3 mL) was added methanesulfonyl chloride (5 μL , 0.06 mmol). HPLC and TLC indicated a complete reaction after 1h. Following removal of organic solvent *in vacuo* the residue was purified by silica flash chromatography (ethyl acetate, neat), affording **SBP-1** as a greasy solid (12 mg, 41 %). ^1H NMR (600 MHz, CD_3CN): δ 7.49 (s, 1H), 7.23 (s, 1H), 6.98 (d, J = 3.5, 1H), 6.73 (s, 1H), 6.12 (d, J = 3.5, 1H), 5.95 (s, 2H), 4.33 (t, 2H), 3.99 (s, 2H), 3.76 (s, 6H), 3.27 (t, 2H), 3.04 (s, 3H), 2.69 (t, 2H), 2.17 (s, 3H), 1.98 (m, 2H), 1.77 (m, 2H), 1.65 (m, 2H), 1.24 (s, 6H); ^{13}C NMR (151 MHz, CD_3CN): δ 158.85, 158.37, 158.20, 157.31, 150.33, 144.42, 140.19, 135.75, 134.15, 134.10, 131.69, 128.97, 115.63, 109.56, 109.36, 106.47, 89.83, 69.59, 68.78, 57.00, 56.28, 40.32, 37.42, 34.19, 32.55, 32.05, 30.81, 29.46, 29.06, 20.48, 18.99; HRESIMS $[\text{M} + \text{H}]^+$ m/z 585.2623 (calcd for $\text{C}_{31}\text{H}_{40}\text{N}_2\text{O}_7\text{S}$, 585.2629).

3-((3,5-dimethoxy-4-(5-((3,3,6-trimethyl-2,3-dihydro-1H-inden-5-yl)oxy)furan-2-carboxamido)phenyl)amino)propyl methanesulfonate (SBP-2). To a stirred solution cooled to 0 °C containing **8b** (18 mg, 0.04 mmol), triethylamine (15 μL , 0.11 mmol) in dichloromethane (3 mL) was added methanesulfonyl chloride (10 μL , 0.13 mmol) under an argon atmosphere. HPLC and TLC indicated a complete reaction after 1 h. Following removal of organic solvent *in vacuo*, the residue was purified by silica flash chromatography (ethyl acetate), affording **SBP-2** as a greasy solid (10 mg, 49 %). ^1H NMR (600 MHz, MeOD): δ 7.11 (d, J = 3.5, 1H), 7.10 (s, 1H), 6.89 (s, 1H), 5.98 (s, 2H), 5.30 (d, J = 3.5, 1H), 4.37 (t, J = 6.1, 2H), 3.77 (s, 6H), 3.29 (t, 2H), 3.07 (s, 3H), 2.86 (t, J = 7.2, 2H), 2.23 (s, 3H), 2.05 (m, 2H), 1.95 (t, J = 7.2, 2H), 1.23 (s, 6H); ^{13}C NMR (151

MHz, MeOD) δ 161.76, 158.51, 153.53, 141.38, 128.33, 127.99, 118.29, 113.86, 90.25, 88.26, 69.62, 56.15, 45.14, 42.86, 40.67, 37.03, 30.36, 30.07, 28.84, 15.88; HRESIMS $[M + H]^+$ m/z 573.2254 (calcd for $C_{29}H_{36}N_2O_8S$, 573.2265).

2-(3,5-dimethoxy-4-(5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamido)phenoxy)ethyl methanesulfonate (SBP-3). To a stirred solution cooled to 0 °C containing **10a** (20 mg, 0.04 mmol), triethylamine (15 μ L, 0.11 mmol) in dichloromethane (3 mL) was added methanesulfonyl chloride (12 μ L, 0.15 mmol) under an argon atmosphere. HPLC and TLC indicated a complete reaction after 1 h. Following removal of organic solvent *in vacuo*, the residue was purified by silica flash chromatography (70% ethyl acetate in hexanes), affording **SBP-3** as a glassy solid (12 mg, 51 %). 1H NMR (600 MHz, $CDCl_3$): δ 7.28 (s, 1H), 7.10 (s, 1H), 7.08 (d, J = 3.1, 1H), 6.88 (s, 1H), 6.19 (s, 2H), 6.01 (d, J = 3.1, 1H), 4.58 (m, 2H), 4.27 (m, 2H), 3.97 (s, 2H), 3.81 (s, 6H), 3.10 (s, 3H), 2.72 (t, J = 6.3, 2H), 2.24 (s, 3H), 1.78 (m, 2H), 1.64 (m, 2H), 1.25 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.47, 157.21, 156.63, 146.96, 143.88, 135.11, 133.34, 132.70, 131.07, 127.97, 116.10, 109.21, 107.50, 91.93, 67.86, 66.39, 56.22, 39.44, 38.04, 33.65, 32.49, 32.00, 30.38, 19.88, 19.03. HRESIMS $[M + H]^+$ m/z 572.2309 (calcd for $C_{30}H_{37}NO_8S$, 572.2313).

2-(3,5-dimethoxy-4-(5-((3,3,6-trimethyl-2,3-dihydro-1H-inden-5-yl)oxy)furan-2-carboxamido)phenoxy)ethyl methanesulfonate (SBP-4). To a stirred solution cooled to 0 °C containing **10b** (20 mg, 0.04 mmol), triethylamine (18 μ L, 0.11 mmol) in dichloromethane (2 mL) was added methanesulfonyl chloride (8 μ L, 0.23 mmol) under an argon atmosphere. HPLC and TLC indicated a complete reaction after 1 h. Following removal of organic solvent *in vacuo*, the residue was purified by silica flash chromatography (80% ethyl acetate in hexanes), affording **SBP-4** as a glassy solid (20 mg, 85 %). 1H NMR (600 MHz, $CDCl_3$): δ 7.19 (s, 1H), 7.12 (d, J = 3.5, 1H), 7.07 (s, 1H), 6.85 (s, 1H), 6.19 (s, 2H), 5.30 (d, J = 3.5, 1H), 4.58 (m, 2H), 4.27 (m, 2H), 3.82 (s, 6H), 3.10 (s, 3H), 2.85 (t, J = 7.2, 2H), 2.26 (s, 3H), 1.94 (t, J = 7.2, 2H), 1.23 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.60, 158.46, 156.66, 152.42, 152.09, 140.07, 139.12, 127.40, 126.83, 117.61, 112.71, 107.61, 92.02, 87.93, 67.86, 66.43, 56.25, 44.27, 41.89, 38.06, 29.70, 28.68, 16.02; HRESIMS $[M + H]^+$ m/z 560.1952 (calcd for $C_{28}H_{33}NO_9S$, 560.1949).

***N*-(4-((3-fluoropropyl)amino)-2,6-dimethoxyphenyl)-5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamide (SB1).** To a stirred vial charged with acid **3** (4 mg, 0.01 mmol) HATU (5 mg, 0.01 mmol), DIPEA (*N,N*-diisopropylethylamine (7 μ L, 0.04 mmol) in DMF (1 mL) allowed to stand for 3 min before amine **7d** (4 mg, 0.02 mmol) was added. HPLC indicated a complete reaction (full consumption of acid) after 1 h. The reaction mixture was diluted with water containing 0.05% TFA and purified by reversed-phase preparative HPLC (isocratic 70 % solvent A at 3.5 ml min⁻¹), and the fraction containing pure **SB1** was isolated by lyophilization, affording a white solid, (2.1 mg, 4.1 μ mol, 41%). ¹H NMR (600 MHz, CD₃CN) δ 7.49 (s, 1H), 7.23 (s, 1H), 6.95 (d, *J* = 3.1, 1H), 6.87 (s, 1H), 6.12 (d, *J* = 3.1, 1H), 5.94 (s, 2H), 4.62 (t, *J* = 5.8, 2H), 4.54 (t, *J* = 5.8, 2H), 3.99 (s, 2H), 3.71 (s, 6H), 3.26 (t, *J* = 6.9, 2H), 2.70 (t, *J* = 6.4, 2H), 2.24 (s, 3H), 2.01 (m, 2H), 1.77 (m, 2H), 1.65 (m, 2H), 1.25 (s, 6H). ¹³C NMR (151 MHz, CD₃CN) δ 158.32, 158.13, 150.47, 147.99, 144.37, 135.70, 134.10, 134.04, 131.61, 128.91, 115.57, 109.30, 89.70, 83.70, 82.63, 46.86, 39.97, 34.13, 32.49, 31.98, 30.75, 20.42, 18.93; HRESIMS [*M* + *H*]⁺ *m/z* 509.2804 (calcd for C₃₀H₃₇FN₂O₄, 509.2810).

***N*-(4-((3-fluoropropyl)amino)-2,6-dimethoxyphenyl)-5-((3,3,6-trimethyl-2,3-dihydro-1*H*-inden-5-yl)oxy)furan-2-carboxamide (SB2).** To a stirred vial charged with acid **9** (11 mg, 0.04 mmol) HATU (15 mg, 0.04 mmol), DIPEA (*N,N*-diisopropylethylamine) (20 μ L, 0.12 mmol) in DMF (300 μ L) allowed to stand for 3 min before amine **7d** (8 mg, 0.04 mmol) was added. HPLC indicated a complete reaction (full consumption of acid) after 1 h. The reaction mixture was diluted with water containing 0.05% TFA and purified by reversed-phase preparative HPLC (isocratic 70 % solvent A at 3.5 ml min⁻¹), and the fraction containing pure **SB2** was isolated by lyophilization affording a white solid, (5 mg, 10 μ mol, 25%). ¹H NMR (600 MHz, CD₃CN): δ 7.42 (bs, 1H), 7.12 (s, 1H), 6.99 (bs, 1H), 6.95 (s, 1H), 5.97 (s, 2H), 5.36 (d, *J* = 3.5, 1H), 4.61 (t, *J* = 5.8, 2H), 4.54 (t, *J* = 5.8, 2H), 3.72 (s, 6H), 3.27 (t, *J* = 6.9, 2H), 2.86 (t, *J* = 7.2, 2H), 2.24 (s, 3H), 1.99 (m, 2H), 1.21 (s, 6H); ¹³C NMR (151 MHz, CD₃CN): δ 160.46, 158.22, 153.32, 153.04, 141.08, 128.15, 127.65, 113.76, 90.37, 88.66, 83.72, 82.65, 56.33, 44.82, 42.40, 30.79, 30.66, 30.08, 28.64, 15.90; HRESIMS [*M* + *H*]⁺ *m/z* 497.2442 (calcd for C₂₈H₃₃FN₂O₅, 497.2447).

N-(4-(2-fluoroethoxy)-2,6-dimethoxyphenyl)-5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxamide (SB3). To a stirred vial charged with acid **3** (5 mg, 0.02 mmol, 1 eq.) HATU (6.1 mg, 0.02 mmol, 1 eq), DIPEA (*N,N*-diisopropylethylamine (14 μ L, 0.08 mmol, 4 eq.) in DMF (1 mL) was allowed to stand for 3 min before amine **7b** (3.5 mg, 0.02 mmol) was added. HPLC indicated a near complete reaction (full consumption of acid) 1h. The reaction mixture was diluted with water containing 0.05% TFA and purified by reversed-phase preparative HPLC (isocratic 70 % solvent A at 3.5 ml min⁻¹), and the fraction containing pure **SB3** was isolated by lyophilization, affording a white solid, (5.4 mg, 10.1 μ mol, 50%). ¹H NMR (600 MHz, CDCl₃): δ 7.39 (bs, 1H), 7.12 (s, 1H), 7.10 (bs, 1H), 6.88 (s, 1H), 6.20 (s, 2H), 6.02 (d, *J* = 3.1, 1H), 4.80 (m, 1H), 4.72 (m, 1H), 4.25 (m, 1H), 4.20 (m, 1H), 3.97 (s, 2H), 3.80 (s, 6H), 2.72 (t, *J* = 6.3, 2H), 2.24 (s, 3H), 1.79 (m, 2H), 1.64 (m, 2H), 1.25 (s, 6H); ¹³C NMR (151 MHz, CDCl₃): δ 159.25, 156.58, 143.93, 135.19, 133.33, 132.53, 131.12, 127.99, 116.96, 109.43, 91.82, 82.52, 81.39, 67.66, 67.53, 56.13, 39.43, 33.65, 32.51, 32.00, 30.39, 19.88, 19.03; HRESIMS [*M* + *H*]⁺ *m/z* 496.2490 (calcd for C₂₉H₃₄FNO₅, 496.2494).

N-(4-(2-fluoroethoxy)-2,6-dimethoxyphenyl)-5-((3,3,6-trimethyl-2,3-dihydro-1*H*-inden-5-yl)oxy)furan-2-carboxamide (SB4). To a stirred vial charged with acid chloride of **9** (6.1 mg, 0.02 mmol) and triethylamine (10 μ L, 0.08) in dry dichloromethane (1.5 mL) at 0 °C amine **7b** (4 mg, 0.02 mmol) in dichloromethane (0.5 mL) was added dropwise. After 1 h the organic solvent was removed *in vacuo*, the residue was taken up in a mixture of water containing 0.05% TFA/acetonitrile (1:1) and purified by reversed-phase preparative HPLC (isocratic 70 % solvent A at 3.5 ml min⁻¹), and the fraction containing pure **SB4** was isolated by lyophilization, affording a white solid, (3.2 mg, 7 μ mol, 33%). ¹H NMR (600 MHz, CDCl₃): δ 7.33 (bs, 1H), 7.18 (s, 1H), 7.07 (bs, 1H), 6.86 (s, 1H), 6.20 (s, 2H), 5.30 (d, *J* = 3.6, 1H), 4.80 (m, 1H), 4.73 (m, 1H), 4.25 (m, 1H), 4.20 (m, 1H), 3.82 (s, 6H), 2.86 (t, *J* = 7.2, 2H), 2.25 (s, 3H), 1.94 (t, *J* = 7.2, 2H), 1.23 (s, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 159.29, 156.58, 152.49, 151.83, 140.30, 127.45, 126.89, 118.96, 112.87, 91.77, 88.00, 82.52, 81.38, 67.64, 56.12, 44.27, 41.85, 29.71, 28.67, 16.02; HRESIMS [*M* + *H*]⁺ *m/z* 484.2129 (calcd for C₂₇H₃₀FNO₆, 484.2130).

Results and Discussion

Synthesis of the indanol furoric acid **9** has been previously described [15]. Synthesis of tetralin **1** from β -ionone with a catalytic amount of iodine was performed using a procedure reported by Parlow *et al.* [14]. The subsequent Friedel-Craft alkylation of **1** with commercial available methyl 5-(chloromethyl)-2-furate using AlCl_3 in dichloroethane has previously been reported to be selective for the C7-carbon in tetraline **1** (Scheme 1) [16]. However, in our hands, the two regioisomers **2a** and **2b** were obtained in roughly equal amounts. Following alkaline hydrolysis of the methyl esters to their corresponding acids gave baseline separation by preparative reversed phased HPLC, obtaining the desired regioisomer **3** in >95 % purity (Scheme 1). With pure **3** at hand, we next turned our attention to the synthesis of intermediate **5**.

Scheme 1.

Nitration of commercial bromo-3,6-dimethoxybenzene (**4**) according to a protocol by Vernier *et al.* in a mixture of nitric acid-acetic anhydride afforded the 5-bromo-1,3-dimethoxy-2-nitro-benzene isomer, **5**, in modest 10 % yield after silica gel chromatography (Scheme 2) [13]. In our previously-reported synthesis of furamide GnRH-R antagonists containing a pyrimidine moiety, displacement of the halogen in 2-chloro-4,6-dimethoxy-5-nitropyrimidine with 2-*tert*-butoxyethoxide proceeded readily at $-42\text{ }^\circ\text{C}$. However, such an approach synthetic approach was not likely to work with the deactivated aromatic system.

Scheme 2.

Consequently, we used a modified Buchwald-Hartwig cross-coupling chemistry. Due to the base-labile nitro group in **5**, we resorted to using Cs_2CO_3 in place of the more commonly employed sodium *tert*-butoxide (NaOtBu). Reaction of **5** with 3-amino-1-propanol catalyzed by the bidentate ligand 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (($\pm$)-BINAP) in conjunction with Pd(II) acetate afforded the desired product in 58 % isolated yield, **6e**, after reaction at $110\text{ }^\circ\text{C}$ overnight under an argon atmosphere (Scheme 2). $^{13}\text{C}/^1\text{H}$ -NMR spectroscopy confirmed that the reaction conditions gave exclusively the

N-arylated product. This constitutes to the best of our knowledge the first reported instance of a selective *N*-arylation with unprotected amino alcohols using palladium-based Buchwald-Hartwig chemistry (Supplemental information). In an analogous fashion, the fluorinated **6f**, was obtained using 3-fluoropropylamine. The same conditions with 2-aminoethanol afforded no product, and further efforts towards a synthesis of the *N*-arylated 2-aminoethanol intermediate were not pursued.

Synthesis of the aryl ether, **6b**, was inspired by previous work on palladium-catalyzed C-O coupling reactions [17]. Gowrisankar and co-workers have reported the use of a bulky modified bippyphos ligand 5-[bis(1-adamantyl)phosphino]-1',3',5'-triphenyl-1,4'-bi-1H-pyrazole(adamantyl-BippyPhos, **L6**) for palladium catalyzed C-O couplings of primary alcohols with an variety of aryl bromides and chlorides [18]. The C-O coupling using ligand **L6** of 2-*tert*-butoxyethanol with the aryl bromide **5** in conjunction with Pd(OAc)₂ in toluene at 110 °C gave the desired aryl ether **6b** in 75 % yield (Scheme 2). Although the same procedure with 2-fluoroethanol was unsuccessful; the alkylfluoride **6d** could be obtained from **6b** by deprotecting the alcohol, yielding **6c**, and its subsequent reaction with diethylaminosulfur trifluoride (DAST) in methylene chloride (DCM).

Scheme 3.

The nitro-group in **6b-f** was reduced with elemental iron to give amines **7a-d**. HATU-mediated coupling of either **3** or **9** with amines **7b** and **7d** yielded the fluorinated reference standard furamide GnRH-R ligands, **SB1-4**. In analogous fashion, precursors for radiolabeling with [¹⁸F]fluoride, **SBP1-4** were derived from reaction of **3** and **9** with amines **7a** and **7c**, obtaining **8a-d**. Of these, **8a** and **b** could be treated directly with methanesulfonyl chloride to yield the precursors **SBP1** and **2**, while **8c** and **d** were deprotected to liberate the alcohol prior to reaction with methanesulfonyl chloride, yielding precursors **SBP3** and **4**. We note that the amine **7c** is unstable, and must be used immediately (Scheme 3).

Binding affinity *in vitro*.

We tested the fluorinated furamide GnRH-R antagonist reference standards **SB1-4** as competitors against the binding of the peptide agonist ¹²⁵I-triptorelin to human GnRH-R

expressed on membranes from Chem-1 cells and rat GnRH-R in HEK293 cells. It is well-established that small molecule GnRH-R antagonists of different scaffolds can show a pronounced interspecies variation in binding affinity [19], sometimes differing by several orders of magnitude for rodent and primate GnRH-Rs. Although this phenomenon generally precludes the use of rodent species in preclinical models, the present furamide-based GnRH-R antagonists are reportedly less stringent with respect to inter-species differences in binding affinity [20]. In agreement with this, our furamides exhibited low nanomolar affinity for the displacement of ^{125}I -triptorelin from both rat and human GnRH-R *in vitro*. Compound **SB3** was particularly potent with, a subnanomolar K_i with respect to human GnRH-R. ^{125}I -Triptorelin displacement results are summarized in Table 2, and representative sets of displacement curves are shown in Fig. 2.

Table 2.

Figure 2.

Radiochemistry.

Radiolabeling was conducted with no-carrier added [^{18}F]fluoride. Reactive $\text{K}[^{18}\text{F}]/\text{K222}$ was prepared and azeotropically dried with anhydrous CH_3CN as previously described [9]. The radiolabeling was initially investigated using 1 mg portions of precursors in 300 μL dimethyl sulfoxide (DMSO) with reaction at 100 $^\circ\text{C}$ for 10 min. The reaction mixtures were then diluted with water (2 mL), and analyzed by reversed phase radio-HPLC, with a second analysis upon spiking with the corresponding reference standards for identification using UV detection at 254 nm. This synthetic methodology was adopted for all four radioligands. Based on radio-HPLC, radiochemical yields ranged from 6 to 16 % among the four ^{18}F -ligands; in order to spare valuable precursor for small animal PET studies described below, we did not attempt any optimization of yield.

The radiosynthesis for *in vitro* and *in vivo* studies used the same radiolabeling conditions as above, typically starting with 37 GBq of [^{18}F]fluoride. After 10 min reaction, free [^{18}F]fluoride was removed from the reaction mixture using an alumina N Sep-Pak column followed by HPLC purification. After dilution of the fractions containing purified

radiotracers with water, they were immobilized on a tC18 Plus Sep-Pak cartridge, rinsed with sterile water, eluted with 1 mL absolute ethanol, and formulated in sterile isotonic saline (final ethanol concentration $\leq 15\%$) for use in the subsequent studies. The aniline-type containing compounds, [^{18}F]SB1 and [^{18}F]SB2, showed a declining radiochemical purity (RCP), which was $\leq 90\%$ after period of two hours at room temperature, likely due to autoradiolysis, as has been reported previously for other aniline-type PET radiopharmaceuticals [21]. We alleviated this by adopting a mobile phase containing 0.5% ascorbic acid in the aqueous system for the preparative HPLC purification, and by further rinsing with 0.5% ascorbic acid/water in the subsequent solvent exchange step. Non-decay corrected yields and RCP of isolated products are given in Table 3. Specific activities were in the range of 300-400 MBq/nmol at end of synthesis, as determined from calibration curves based on the reference standards. A combined radiochromatogram of [^{18}F]SB1, 2, 3 and 4 is shown in Fig. 3.

Table 3.

Figure 3.

Determination of the partition coefficient.

The measured distribution coefficient (log D) for [^{18}F]SB1, 2, 3 and 4 were 1.43, 1.51, 1.85 and 2.10, respectively. The values were lower than expected from the log P-values calculated using ChemDraw professional 15 software (Table 1.) The presence of one or more hydrophilic impurities could be a factor that would lead to an underestimation of the determined log P-values. Nevertheless, the log D values of the synthesized ^{18}F -GnRH antagonists are in an interval typically seen for PET tracers capable of crossing the BBB [22, 23].

Ex vivo and in vivo stability.

Stability of the ^{18}F -labeled compounds in rat serum was investigated in vitro after at 1 and 2 h time points. All four radiotracer displayed good stability with little or no signs of degradation. The in vivo blood stability in rats of the four small molecule ^{18}F -GnRH antagonists, [^{18}F]SB1-4 was studied in an analogous manner; tracers were injected in

living rats and blood was collected and analyzed by radio-HPLC after 1 h. All four PET ligands (Table 4) remained nearly intact in blood to 1 h postinjection, with the great majority of activity remaining as the parent compounds (98-80% intact). [^{18}F]SB1 and 3 were seemingly more prone to blood metabolism, with around 20% loss in purity. This stability is an important precondition for our use of the IDIF (*vide infra*) for quantitation of uptake kinetics. Urine was collected after 1 h as a part of the biodistribution studies. All tracers appeared to be intact in the excreted urine, except for [^{18}F]SB3, with only 19 % of the total collected radioactivity associated with intact tracer. We speculate that the HPLC of [^{18}F]SB3 failed, accounting this discrepancy compared to the other radiotracers.

Biodistribution *ex vivo* and PET studies.

Biodistribution data in naive male rats for [^{18}F]SB1-4 60 min p.i are shown in Table 5. The data obtained for the tracers at the 1 h time-point revealed an overall similar biodistribution for all four compounds. The tracers cleared relatively rapidly from the blood (declining to 0.06–0.011 %ID g⁻¹) with low retention in bone, indicating little or no defluorination *in vivo*. Excretion of the tracers was distributed relative uniformly via both the renal and hepatobiliary pathways, as revealed by the high percentage of injected activity found in both urine/kidneys and feces/liver. The cerebral uptake at one hour p.i. was low for all tracers, with brain uptake of the slightly more lipophilic SB3 and SB4 being marginally higher than SB1-2, 0.6 vs. 0.4 % ID g⁻¹, respectively.

Table 4.

Table 5.

Given the documented near absence of [^{18}F]-labeled metabolites in blood, we determined that an image derived arterial input function (IDIF) should be valid for a more quantitative approach to tracer pharmacokinetics, based on linear graphic analysis methods reported and validated previously [11]. Parametric maps (Fig. 5.) show the uptake of [^{18}F]SB3 (left hand column) and [^{18}F]SB4 (right hand column) expressed in (A) SUV units of %ID g⁻¹ of brain in the initial three minutes of the emission recording, (B)

%ID g⁻¹ in the interval of 45-90 minutes, and (C) calculated as equilibrium distribution volume (V_T ; ml g⁻¹) from the entire dynamic recording relative to an IDIF measured in the left ventricle of the heart. PET results projected upon an MR template of rat brain illustrations of the initial distribution showed uniform cerebral uptake of [¹⁸F]SB3 and 4, and late frames showed homogeneous distribution, with no evidence of relatively increased retention in rat hippocampus of these or any of the four PET tracers. We estimated the apparent plasma volumes (V_p ; ml g⁻¹) and unidirectional blood brain clearances K_I (ml g⁻¹ min⁻¹) of the four tracers in rat forebrain from the first two minutes of the data recordings, and the 1-tissue compartment (1-TCM) parameters in hippocampus using the entire 120 minute recordings (Table 6). Apparent plasma volumes tended to exceed the canonical value of 0.05 ml g⁻¹; to linear graphical analysis, the magnitude of K_I was about two-fold higher for [¹⁸F]SB3 and 4 (approximately 0.04 ml g⁻¹ min⁻¹) than for [¹⁸F]SB1 and 2, and was systematically lower when calculated by the 1TCM analysis of the prolonged recordings, as expected for tracers with rapid clearance (high k_2). V_T values were less than 1 ml g⁻¹, with no evidence in the parametric maps of enrichment in the hippocampus or other regions with abundant GnRH-R, confirming the qualitative results in Figure 5.

4. Conclusion

[¹⁸F]SB1-4, novel high affinity small molecule ¹⁸F-fluorinated GnRH-R antagonist permeable to the BBB, were evaluated. We cannot presently account for the failure of the compounds to detect specific binding in the hippocampus, but suggest that the age of the rats, the low cerebral B_{max} of GnRH-R in rats, and lower *in vivo* vs. *in vitro* GnRH-R affinity may be factors to consider in future studies of this type. The molecular size of [¹⁸F]SB1-4 and their relatively high polar surface, bordering the upper limits typically observed for most PET CNS tracers, might account for the low brain penetration. In addition, the association kinetics for binding of these ligands to the GnRH-R (k_{on}) are unknown, such that washout from brain may occur prior to substantial specific binding can occur. In particular, we intend to investigate the effects of blocking P-glycoprotein (Pgp), a parameter that can strongly influence the brain efflux of many compounds. Moreover, further studies in a non-rodent model, e.g. nonhuman primates, could provide

vital data for the further applicability of these novel tracers. Investigations of the cerebral GnRH-R binding of these tracers using autoradiography *in vitro* are also warranted, given that no small molecular radioligand for such studies is currently available. Finally, we conclude given the high affinity and observable, albeit low, brain uptake, this class of compounds should facilitate further studies as cerebral GnRH-R tracers.

Acknowledgements

This research was supported in part by the Office of Science, United States Department of Energy Grant (DE-SL0002061), the Stiftelsen Kristian Gerhard Jebsen Foundation, Norwegian Research Council, HSØ (South-Eastern Norway Regional Health Authority) Innovation Grant and Norwegian Cyclotron Centre Grant. Many thanks to Thor Audun Saga for much appreciated support. We also extend our thanks to David L. Kukis, Lina Planutyte, Leah Knight and Jennifer Fung at CMGI (BME, UC Davis) for excellent technical assistance.

References

- [1] Pawson AJ, Faccenda E, Maudsley S, Lu Z-L, Naor Z, and Millar RP. Mammalian Type I Gonadotropin-Releasing Hormone Receptors Undergo Slow, Constitutive, Agonist-Independent Internalization. *Endocrinology* 2008;149:1415-22.
- [2] Cui J, Smith RG, Mount GR, Lo J-L, Yu J, Walsh TF, et al. Identification of Phe³¹³ of the Gonadotropin-Releasing Hormone (GnRH) Receptor as a Site Critical for the Binding of Nonpeptide GnRH Antagonists. *Molecular Endocrinology* 2000;14:671-81.
- [3] Harrison GS, Wierman ME, Nett TM, and Glode LM. Gonadotropin-releasing hormone and its receptor in normal and malignant cells. *Endocrine-Related Cancer* 2004;11:725-48.

- [4] Skinner DC, Albertson AJ, Navratil A, Smith A, Mignot M, Talbott H, et al. Effects of Gonadotrophin-Releasing Hormone Outside the Hypothalamic-Pituitary-Reproductive Axis. *Journal of Neuroendocrinology* 2009;21:282-92.
- [5] Leblanc P, Crumeyrolle M, Latouche J, Jordan D, Fillion G, L'Heritier A, et al. Characterization and Distribution of Receptors for Gonadotropin-Releasing Hormone in the Rat Hippocampus. *Neuroendocrinology* 1988;48:482-8.
- [6] Albertson AJ, Navratil A, Mignot M, Dufourny L, Cherrington B, and Skinner DC. Immunoreactive GnRH type I receptors in the mouse and sheep brain. *Journal of Chemical Neuroanatomy* 2008;35:326-33.
- [7] Amory J, Leonard T, Page S, O'Toole E, McKenna M, and Bremner W. Oral administration of the GnRH antagonist acyline, in a GIPET®-enhanced tablet form, acutely suppresses serum testosterone in normal men: single-dose pharmacokinetics and pharmacodynamics. *Cancer Chemother Pharmacol* 2009;64:641-5.
- [8] Caraty A and Skinner DC. Gonadotropin-Releasing Hormone in Third Ventricular Cerebrospinal Fluid: Endogenous Distribution and Exogenous Uptake. *Endocrinology* 2008;149:5227-34.
- [9] Olberg DE, Andressen KW, Levy FO, Klaveness J, Haraldsen I, and Sutcliffe JL. Synthesis and in vitro evaluation of small-molecule [18F] labeled gonadotropin-releasing hormone (GnRH) receptor antagonists as potential PET imaging agents for GnRH receptor expression. *Bioorganic & Medicinal Chemistry Letters* 2014;24:1846-50.
- [10] Heitman LH, Ye K, Oosterom J, and IJzerman AP. Amiloride Derivatives and a Nonpeptidic Antagonist Bind at Two Distinct Allosteric Sites in the Human Gonadotropin-Releasing Hormone Receptor. *Molecular Pharmacology* 2008;73:1808-15.

- [11] la Fougère C, Böning G, Bartmann H, Wängler B, Nowak S, Just T, et al. Uptake and binding of the serotonin 5-HT_{1A} antagonist [18F]-MPPF in brain of rats: Effects of the novel P-glycoprotein inhibitor tariquidar. *NeuroImage* 2010;49:1406-15.
- [12] Badr M and Pelletier G. Characterization and autoradiographic localization of LHRH receptors in the rat brain. *Synapse* 1987;1:567-71.
- [13] Vernier JM, Delarosa M, Chen H, Wu JZ, Larson GL, and Cheney LW. Derivatives of 4-(n-azacycloalkyl) anilides as potassium channel modulators. 2011.
- [14] Parlow JJ. Selective syntheses of substituted 6-alkyl-1,1-dimethyl-1,2,3,4-tetrahydronaphthalenes. *Tetrahedron* 1993;49:2577-88.
- [15] Li H, Anderes KL, Kraynov EA, Luthin DR, Do Q-Q, Hong Y, et al. Discovery of a Novel, Orally Active, Small Molecule Gonadotropin-Releasing Hormone (GnRH) Receptor Antagonist. *Journal of Medicinal Chemistry* 2006;49:3362-7.
- [16] Anderson MBU, Christie, Lance C. (US), Feng, Jun (US), Hong, Yufeng (US), Haitao LI. (US), Pathak, Ved P. (US), Rajapakse, Ranjan (US), Tompkins, Eileen (US), Vazir, Haresh N. (US). Non-peptide compounds affecting the action of gonadotropin-releasing hormone (GnRH). Pfizer (US); 2003.
- [17] Schlummer B and Scholz U. Palladium-Catalyzed C-N and C-O Coupling—A Practical Guide from an Industrial Vantage Point†. *Advanced Synthesis & Catalysis* 2004;346:1599-626.
- [18] Gowrisankar S, Sergeev AG, Anbarasan P, Spannenberg A, Neumann H, and Beller M. A General and Efficient Catalyst for Palladium-Catalyzed C–O Coupling Reactions of Aryl Halides with Primary Alcohols. *Journal of the American Chemical Society* 2010;132:11592-8.

- [19] Betz SF, Zhu Y-F, Chen C, and Struthers RS. Non-Peptide Gonadotropin-Releasing Hormone Receptor Antagonists. *Journal of Medicinal Chemistry* 2008;51:3331-48.
- [20] Sarma PKS, Tiwari A, Kondaskar A, and Cliffe IA. Peptidomimetic GnRH receptor antagonists for the treatment of reproductive and proliferative diseases. *Expert Opinion on Therapeutic Patents* 2006;16:733-51.
- [21] Scott PJH, Hockley BG, Kung HF, Manchanda R, Zhang W, and Kilbourn MR. Studies into radiolytic decomposition of fluorine-18 labeled radiopharmaceuticals for positron emission tomography. *Applied Radiation and Isotopes* 2009;67:88-94.
- [22] Pike VW. PET Radiotracers: crossing the blood-brain barrier and surviving metabolism. *Trends in pharmacological sciences* 2009;30:431-40.
- [23] Ogawa K, Shiba K, Akhter N, Yoshimoto M, Washiyama K, Kinuya S, et al. Evaluation of radioiodinated vesamicol analogs for sigma receptor imaging in tumor and radionuclide receptor therapy. *Cancer Science* 2009;100:2188-92.

Schemes and Figures

Scheme 1. Synthesis and purification of 5-((3,8,8-trimethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)furan-2-carboxylic acid (**3**)

Scheme 2. Synthesis of aryl intermediates **7a-d**.

Scheme 3. Synthesis of reference standards **SB1-4** and precursors **SBP1-4**

Figure 1. Structure of the two previously synthesized ^{18}F -radiolabelled furamide GnRH antagonists.

IP1 $R_1 = ^{18}\text{F}$
IP2 $R_1 = \text{O}(\text{CH}_2)_2 ^{18}\text{F}$

Figure 2. Representative displacement curves for the GnRH-R agonist ligand [^{125}I]triptorelin by the four small molecule GnRH antagonists **SB1-4** *in vitro* with respect to human (left) and rat (right) GnRH-R.

Figure 3. Combined radio-HPLC chromatogram of purified [^{18}F]SB1-4. Note the correlation of retention time (t_R) and predicted log P (Table 1.)

Figure 4. Linear graphic analysis (Gjedde-Patlak) of the distribution volume (V_d) of [^{18}F]SB3 in forebrain of a rat relative to the normalized heart ventricle image-derived input function (Theta) measured by PET during 120 minutes of laboratory time. The initial slope (blue line) corresponds to unidirectional blood-brain clearance (K_I ; $0.04 \text{ ml g}^{-1} \text{ min}^{-1}$), with ordinate intercept corresponding to the apparent plasma volume (V_p ; 0.1 ml g^{-1}). The magnitude of V_d later approaches an asymptote suggesting an equilibrium distribution volume (V_T) of approximately 0.8 ml g^{-1} .

Figure 5. Voxel-wise parametric maps showing the mean uptake in %ID ml⁻¹ units of [¹⁸F]SB3 and [¹⁸F]SB4 during (A) the initial two minutes of the PET recording, (B) the final 75 minutes of the PET recording, and (C) the equilibrium distribution volume (V_T ; ml g⁻¹) calculated by a 1-tissue compartment model relative to the image-derived input function for the entire 120 min PET recording.

Table 1. Molecular weight and physiochemical parameters for the small molecule fluorinated GnRH antagonists **SB1-4** described in this work, compared to the pyrimidine based agonist **IP1-2** previously reported.

Compound	Mw (Da)	tPSA ^a	log P ^b	Log D (oct/PBS pH 7.4) ^c
SB1	508.6	68.2	4.25	1.43±0.17
SB2	496.6	78.1	4.53	1.51±0.12
SB3	495.6	66.0	4.52	1.85±0.06
SB4	483.5	75.3	4.80	2.10±0.40
IP1	485.5	99.9	4.81	1.33 [*]
IP2	441.5	90.7	4.66	1.74 [*]

^{a,b} Values calculated using ChemDraw Professional 15

^c Experimentally determined Log P values, i.e. octanol:water partition coefficient

^{*} Values obtained from reference 6.

Table 2. p*K_i* with SEM and *K_i* values obtained for **SB1-4** for human and rat GnRH-R (n = 3).

Ligand	Human GnRH-R		Rat GnRH-R	
	p <i>K_i</i> (± SEM)	<i>K_i</i> (nM)	p <i>K_i</i> (± SEM)	<i>K_i</i> (nM)
SB1	8.31 (± 0.02)	4.9	8.91 (± 0.08)	1.2
SB2	8.94 (± 0.24)	1.1	8.99 (± 0.22)	1.0
SB3	9.52 (± 0.30)	0.3	8.90 (± 0.07)	1.3
SB4	8.87 (± 0.40)	1.3	8.43 (± 0.18)	3.7

Table 3. Radiochemical purity (RPC) for purified and formulated tracer, yield relative to starting activity of [¹⁸F]fluoride, and retention times (*R_t*) of radiolabeled compounds [¹⁸F]**SB1-4**.

Compound	RCP (%)	Yield (%) ¹	HPLC <i>R_t</i> (min)
[¹⁸ F] SB1	97.8	5.5	17.3
[¹⁸ F] SB2	99.3	16.0	16.2
[¹⁸ F] SB3	99.7	8.4	19.3
[¹⁸ F] SB4	98.3	12.0	18.3

¹Calculated from radioactivity at start of synthesis

Table 4. Stability in rat serum *in vitro* (at 1 and 2 h), *in vivo* blood and urine stability (at 1 h) as determined by radio-HPLC.

Compound	% Intact ¹⁸ F-compound			
	Serum 1 h	Serum 2 h	Blood 1h	Urine 1h
[¹⁸ F] SB1	> 90	88.5	81.0	94.5
[¹⁸ F] SB2	99.1	97.5	97	99

$[^{18}\text{F}]\text{SB3}$	> 95	91.1	88.1	18.5
$[^{18}\text{F}]\text{SB4}$	99.0	97.3	97.5	99.0

Table 5. Biodistribution of [^{18}F]SB1-4 1 h postinjection presented as %ID g^{-1} . Each estimate is the mean (SD) of the three determinations.

Organ/tissue	Percentage for injected dose per gram ($\bar{x} \pm s$, %ID g^{-1})			
	SB1	SB2	SB3	SB4
Urine	2.58 $\pm$ 1.16	2.14 $\pm$ 1.41	2.30 $\pm$ 0.26	2.06 $\pm$ 0.27
Blood	0.07 $\pm$ 0.01	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.11 $\pm$ 0.01
Bladder	0.20 $\pm$ 0.04	0.13 $\pm$ 0.02	0.14 $\pm$ 0.03	0.15 $\pm$ 0.03
Liver	1.05 $\pm$ 0.06	0.66 $\pm$ 0.00	0.63 $\pm$ 0.02	0.57 $\pm$ 0.14
Lungs	0.24 $\pm$ 0.07	0.28 $\pm$ 0.15	0.15 $\pm$ 0.02	0.28 $\pm$ 0.14
Heart	0.36 $\pm$ 0.07	0.23 $\pm$ 0.02	0.17 $\pm$ 0.01	0.24 $\pm$ 0.06
Spleen	0.28 $\pm$ 0.02	0.18 $\pm$ 0.01	0.13 $\pm$ 0.01	0.15 $\pm$ 0.03
Pancreas	0.50 $\pm$ 0.06	0.28 $\pm$ 0.01	0.26 $\pm$ 0.02	0.35 $\pm$ 0.06
Kidney	0.45 $\pm$ 0.03	0.25 $\pm$ 0.01	0.24 $\pm$ 0.01	0.28 $\pm$ 0.04
Small Intestines	0.31 $\pm$ 0.09	0.22 $\pm$ 0.02	0.27 $\pm$ 0.06	0.45 $\pm$ 0.23
Large Intestines	0.24 $\pm$ 0.05	0.15 $\pm$ 0.02	0.11 $\pm$ 0.00	0.17 $\pm$ 0.02
Feces	1.29 $\pm$ 0.25	0.57 $\pm$ 0.10	1.65 $\pm$ 0.47	2.15 $\pm$ 1.24
Testis	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.07 $\pm$ 0.01
Stomach	0.24 $\pm$ 0.02	0.15 $\pm$ 0.01	0.14 $\pm$ 0.01	0.18 $\pm$ 0.05
Skin	0.18 $\pm$ 0.01	0.12 $\pm$ 0.01	0.15 $\pm$ 0.03	0.18 $\pm$ 0.04
Muscle	0.16 $\pm$ 0.01	0.13 $\pm$ 0.01	0.11 $\pm$ 0.02	0.14 $\pm$ 0.03
Bone	0.22 $\pm$ 0.04	0.14 $\pm$ 0.01	0.08 $\pm$ 0.01	0.09 $\pm$ 0.03
Pituitary	0.29 $\pm$ 0.06	0.23 $\pm$ 0.02	0.18 $\pm$ 0.03	0.23 $\pm$ 0.03
Brain	0.04 $\pm$ 0.00	0.04 $\pm$ 0.01	0.06 $\pm$ 0.00	0.06 $\pm$ 0.01

Table 6. Summary of kinetic analysis of PET data showing results of (Gjedde-Patlak) linear graphic analysis of the apparent plasma volume (V_p) and unidirectional blood-brain clearance (K_l) of the four tracers in the forebrain during the first two minutes, and 1-TCM analysis for the bilateral hippocampus, using the entire PET recordings and the image-derived input function.

Comp.	Gjedde-Patlak		1-TCM		
	V_p (ml g^{-1})	K_l (ml $\text{g}^{-1} \text{min}^{-1}$)	K_l (ml $\text{g}^{-1} \text{min}^{-1}$)	k_2 (min $^{-1}$)	V_e (ml g^{-1})
[^{18}F]SB1	0.06	0.021	0.029	0.059	0.48
[^{18}F]SB2	0.07	0.021	0.037	0.087	0.42
[^{18}F]SB3	0.08	0.044	0.049	0.068	0.72
[^{18}F]SB4	0.11	0.035	0.070	0.123	0.56